

Identification of Bioactive Compounds in the acetone extract of *Daedalea elegans* using Gas Chromatography – Mass Spectrometry: A review

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Abstract

Daedalea elegans is a Nigerian wild (non-edible) higher fungus with great potentials in the pharmaceutical, textile, cosmetics and food industry. This current study investigates the bioactive compounds that can be found in the acetone extract of *D. elegans* using Gas Chromatography-Mass Spectrometry (GC-MS). There were twenty-eight compounds identified to be present in the acetone extract of the fungi under study and these are Benzoic acid (0.40%), Nonanoic acid (0.14%), Oxetane, 2,2,4-trimethyl- (0.28%), n-Decanoic acid (0.09%), Phthalimide (0.44%) Dodecanoic acid (0.24%), E-2-Hexenyl benzoate (0.21%), 2,4-Difluorobenzene, 1-benzyloxy- (0.16%), Tetratetracontane (0.55%), Isopropylphosphonic acid, fluoroanhydride (0.28%), Benzene, (1-methylundecyl)- (0.21%), Tetradecanoic acid (0.76%), Cyclohexanepropanol, .alpha.,2,2,6-tetrame (0.56%), Pentadecanoic acid (0.71%), E-2-Hexenyl benzoate (0.32%), Pentadecanoic acid (0.97%), 1-Decanol, 2-hexyl- (0.46%), 9-Tetradecenal, (Z) (1.67), n-Hexadecanoic acid (23.59%) is the second most abundant, Phthalic acid, butyl undecyl ester (1.08%), Eicosanoic acid (0.79%), 9,12-Octadecadienoic acid (Z,Z)- (44.64%) was the highest in quantity, Octadecanoic acid (6.98%), Bis(2-ethylhexyl) phthalate (2.64%), 2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol (1.95%), 9(11)-Dehydroergosteryl benzoate (8.37%), 9(11)-Dehydroergosterolsylate (1.28%), 4,6-Decadienal, 8-ethyl-10-[4-hydroxy-8-(2-hydroxypropyl)-3,9 (0.22%). These compounds possess activities which includes but not limited to cancer chemotherapy, antifungal agent against pine weevil, antifungal agent in topical therapeutic preparation, anti-inflammatory, immunomodulatory, anti-convulsant, antioxidants, hypocholesterolemic, anti-androgenic, nematicide, analgesic, intermediate for food-grade additives, lubricants, greases, rubber, dyes and plastic, antineoplastic agent, biosynthesis of prostaglandins and cell membrane to mention a few. This study has been able to show that *D. elegans* is a good source of bioactive compounds with great potentials that can be harnessed in various industries.

Keywords— acetone extract, Bioactive compounds, *Daedalea elegans*, GC-MS analysis.

I. INTRODUCTION

Daedalea elegans is an inedible mushroom which are highly variable species or cluster of species, perhaps recognized by its elongated, maze-like pores; its thin, tough, whitish to brownish cap with zones of colour; and the tendency of its pore surface to bruise reddish. It is lumpy towards the point of attachment and smoother toward the margin. Wild indigenous mushrooms have been found to be nutritious and very important for medicinal

purposes, many have been used as tea and nutritional food for their special fragrance and texture purposes [1]. Majority are very good sources of amino acids, ascorbic acid, glycogen, lipid, sugar and vitamins and minerals such as calcium, iron, phosphorus, potassium, sodium, magnesium and zinc [2,3,4].

The nutritional values and taste components of many mushrooms was reported by Yang *et al.*, [5]. Also, antimicrobial potentials of many macrofungi have been

studied and reported by several researchers [6,7,8,], however, there are little information on the bioactive compounds of mushroom extracts. *D. elegans* is a Nigerian wild mushroom and has been reported as a source of phytochemicals and antioxidants with medicinal [9,6]. Other Nigerian wild mushrooms have also been reported to have antimicrobial potentials, but little/no study has reported the bioactive compounds conferring these antimicrobial properties. Therefore, it is important to determine bioactive compounds present in the extracts which are responsible for the bioactivity of the mushroom and their medicinal values making the GC-MS analysis inevitable. GC-MS works by combining separation (GC) and identification (MS) techniques, thereby, making it ideal for both quantitative and qualitative analysis of volatile and semi-volatile compounds. The aim of this present study is to screen *D. elegans* for the presence of bioactive compounds using acetone as the solvent for the extraction.

II. METHODOLOGY

Sample collection and identification: *D. elegans* used in this study was collected from different areas (University Botanical Gardens and Nursery Section of Botany and Microbiology Department) within the University of Ibadan campus between September and November 2016 in a sterile polyethylene bag and transported to the Botany Laboratory of the University of Ibadan. The mushroom was immediately identified in the laboratory by their spore prints and by comparing their morphological, anatomical and physiological characteristics with the standard descriptions of Zoberi [10] and that of Alexopolous *et al.*, [11]. This identification was authenticated by Professor S. G. Jonathan, Department of Botany, University of Ibadan, Nigeria. After proper identification was done, the sample was then transported to Babcock University where the experiment was carried out.

1. Preparation of Crude Extract

The fruiting body of the test mushroom was allowed to air dry at room temperature. The dried carpophore was divided into bits and pulverized with a grinding machine. Eighty grams of the powdered sample was soaked in

700ml of acetone in an Erlenmeyer flask. The flasks were covered with cotton wool and aluminum foil and allowed to stand for 48 hours with intermittent shaking. It was filtered through Whatman filter paper no 1 and the sample was re-suspended in solvent and allowed to stand for additional 7 days. This was also filtered through Whatman filter paper no 1. The filtrate obtained was concentrated using rotary evaporator at 50°C, and was further concentrated to remove the remaining acetone using hot air oven at 45°C for 19 hours and was then stored in the refrigerator before further analysis was carried out.

2. GC-MS Analysis:

GC-MS analysis was conducted at Shimadzu Training Center for Analytical Instruments (STC) Lagos. The extracts were analyzed by GCMS-QP2010SE (SHIMADZU, JAPAN) equipped with DB-5MS (0.25µm X 30m X 0.25mm). Helium was used as the carrier gas at a flow rate 0.9ml/min. 1.0µl injection volume, injector temperature was 250°C; ion source temperature was 200°C. Interface temperature was 250°C. Oven temperature was 60°C held for 2min with an increase of 15°C/min to 120°C, ending with 300°C (15°C/min). Mass spectrometer was set to operate in electron ionization mode with an ionizing energy of 70eV as acquisition mass range from 45-700 a.m.u. Total running time was about 30 minutes. Further identification was made by comparison of their mass spectra with those stored in the National Institute of Standards and Technology (NIST) database.

III. RESULTS

Twenty-Eight bioactive compounds were identified in the acetone extract of *D. elegans*. The dry weight of the yield was 0.58g. Compounds were identified from NIST database Library of GC-MS instrument. Identified compounds with their name, retention time and peak (area) percentage are given in Table 1, while the GC-MS chromatogram is shown in Fig. 1.

Only four compounds dominated the acetone extract and these accounts for 83.58 of the entire compounds namely: [9,12- octadecadienoic acid (44.64%), n-hexadecanoic acid (23.59%), 9(11)-dehydroergosteryl benzoate (8.37%), octadecanoic acid (6.98%)].

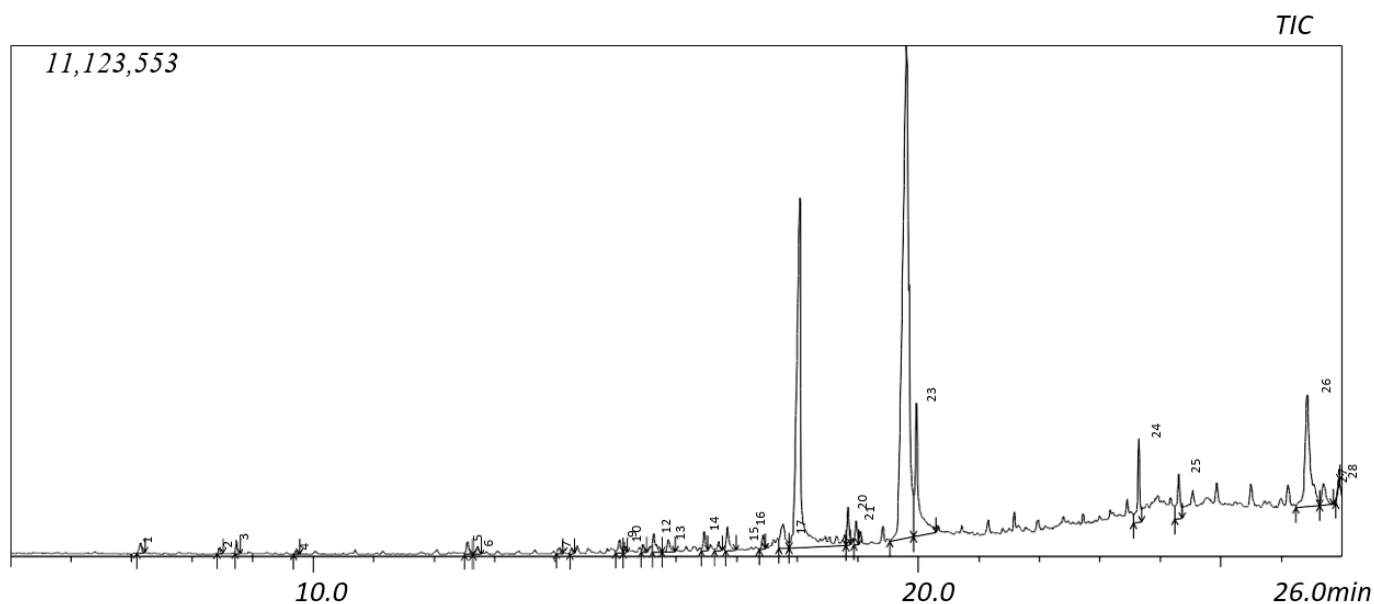


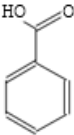
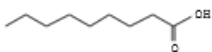
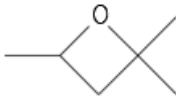
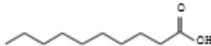
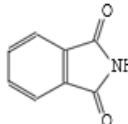

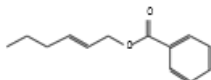
Fig. 1: GC-MS chromatogram of acetone extract of *D. elegans*

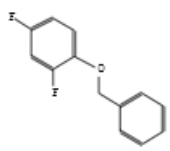

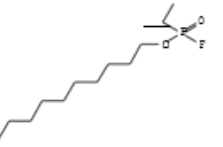
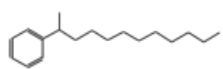
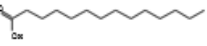
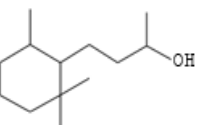
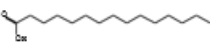
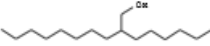
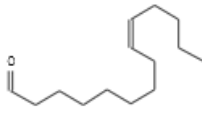
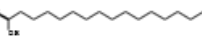
Table 1: GC-MS profiling of acetone extract of *D. elegans*

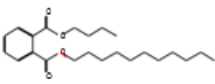

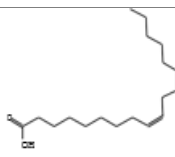
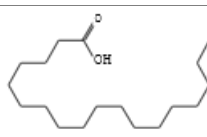
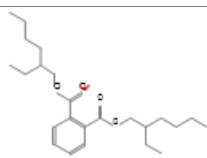
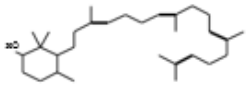
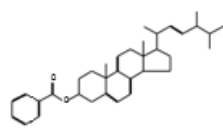
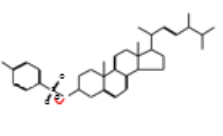
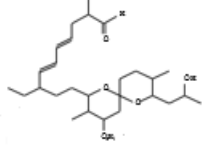
Peak #	R. Time	Molecular Formula	Molecular Weight	Name	Area %
1	7.154	C ₇ H ₆ O ₂	122	Benzoic acid	0.40
2	8.449	C ₉ H ₁₈ O ₂	158	Nonanoic acid	0.14
3	8.734	C ₆ H ₁₂ O	100	Oxetane, 2,2,4-trimethyl-	0.28
4	9.714	C ₁₀ H ₂₀ O ₂	172	n-Decanoic acid	0.09
5	12.551	C ₈ H ₅ NO ₂	147	Phthalimide	0.44
6	12.720	C ₁₂ H ₂₄ O ₂	200	Dodecanoic acid	0.24
7	14.057	C ₁₃ H ₁₆ O ₂	204	E-2-Hexenyl benzoate	0.53
8	14.267	C ₁₃ H ₁₀ F ₂ O	220	2,4-Difluorobenzene, 1-benzyloxy-	0.16
9	15.067	C ₄₄ H ₉₀	618	Tetratetracontane	0.55
10	15.141	C ₁₃ H ₂₈ FO ₂ P	266	Isopropylphosphonicacid, fluoroanhydride	0.28
11	15.453	C ₁₈ H ₃₀	246	Benzene, (1-methylundecyl)-	0.21
12	15.628	C ₁₄ H ₂₈ O ₂	228	Tetradecanoic acid	0.76
13	15.868	C ₁₃ H ₂₆ O	198	Cyclohexanepropanol, .alpha.,2,2,6-tetrame	0.56
14	16.464	C ₁₅ H ₃₀ O ₂	242	Pentadecanoic acid	0.71
15	16.703	C ₁₃ H ₁₆ O ₂	204	E-2-Hexenyl benzoate	0.32
16	16.845	C ₁₅ H ₃₀ O ₂	242	Pentadecanoic acid	1.68
17	17.435	C ₁₆ H ₃₄ O	242	1-Decanol, 2-hexyl-	0.46
18	17.764	C ₁₄ H ₂₆ O	210	9-Tetradecenal, (Z)-	1.67
19	18.045	C ₁₆ H ₃₂ O ₂	256	n-Hexadecanoic acid	23.59
20	18.837	C ₂₃ H ₃₆ O ₄	376	Phthalic acid, butyl undecyl ester	1.08
21	18.972	C ₂₀ H ₄₀ O ₂	312	Eicosanoic acid	0.79

22	19.801	C ₁₈ H ₃₂ O ₂	280	9,12-Octadecadienoic acid (Z,Z)-	44.64
23	19.970	C ₁₈ H ₃₆ O ₂	284	Octadecanoic acid	6.98
24	23.641	C ₂₄ H ₃₈ O ₄	390	Bis(2-ethylhexyl) phthalate	2.64
25	24.302	C ₃₀ H ₅₂ O	428	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-he	1.95
26	26.423	C ₃₅ H ₄₆ O ₂	498	9(11)-Dehydroergosterylbenzoate	8.37
27	26.694	C ₃₅ H ₄₈ O ₃ S	548	9(11)-Dehydroergosteroltosylate	1.28
28	26.948	C ₂₇ H ₄₆ O ₅	450	4,6-Decadienal,8-ethyl-10-[4-hydroxy-8-(2	0.22
					100.00

Table 2: Therapeutic activity of the bioactive compounds identified from the acetone extract of *D.elegans*

Name	Nature of Compound	Chemical Structure	Medicinal Properties/Therapeutic Uses
Benzoic acid	Aromatic acid		Corrosion-inhibitor in emulsions and paints, as well as an anti-freeze formulation, plugging agent, and modifier in oil well applications, a moderately effective preservative providing that the pH of the formulation (medicines, cosmetics, or foods) does not exceed 5.0, antifungal agent in topical therapeutic preparation with salicylic acid [12,13]
Nonanoic acid	Saturated fatty acid		Used in the preparation of plasticizers and lacquers, treating seizures, antifeedant against pine weevil [14,15]
Oxetane, 2,2,4-trimethyl-	Volatile organic		Cancer chemotherapy [16]
n-Decanoic acid	Saturated fatty acid		Used in the manufacture of esters for artificial fruit flavors and perfumes, and as an intermediate for food-grade additives, lubricants, greases, rubber, dyes, plastics (PubChem CID 2969).
Phthalimide	Organic		Anti-convulsant, anti-inflammatory, analgesic, hypolipidemic and immunomodulatory activities [17,18]
Dodecanoic acid	Saturated fatty acid		Used in many soaps and shampoos, flavouring agents, plant growth regulator (PubChem CID 3893)
E-2-Hexenyl benzoate			Reverse Transcriptase inhibitor [19]

2,4-Difluorobenzene,1-benzyloxy-			Not found
Tetratetracontane	Alkane		It has a role as a human metabolite (PubChem CID 23494)
Isopropylphosphonic acid, fluoroanhydride	Anhydride		Increase aromatic amino acid decarboxylase activity, inhibit production of uric acid [19]
Benzene, (1-methylundecyl)-	Volatile aromatic hydrocarbon		Antineoplastic agent, for chemically bleaching and whitening the skin (PubChem CID 17628)
Tetradecanoic acid			Antibacterial, Antioxidative [20]; Lubricant, Cosmetic, Cancer preventive [19]
Cyclohexanepropanol, alpha.,2,2,6-tetramethyl			Used as fragrance in cosmetic products [19]
Pentadecanoic acid	Saturated fatty acid		It has a role as a plant metabolite, a food component, a <i>Daphnia magna</i> metabolite, a human blood serum metabolite and an algal metabolite (PubChem CID 13849) improved insulin sensitivity [21] and reduced risk for type 2 diabetes [22]
1-Decanol, 2-hexyl-			Not found
9-Tetradecenal, (Z)-			Increase zinc bioavailability[19]
n-Hexadecanoic acid			Anti-inflammatory, antioxidant, hypocholesterolemic, flavor, nematocide, pesticide, anti-androgenic [23,24,25]

Phthalic acid, butyl undecyl ester			Increase aromatic amino acid decarboxylase activity, inhibit production of uric acid, urinary acidulant [19]
Eicosanoic acid			Not found
9,12-Octadecadienoic acid (Z,Z)-	Linolenic acid		Biosynthesis of prostaglandins and cell membranes [25] anti-inflammatory, hepatoprotective, anti-arthritis, anti-histamine [23]
Octadecanoic acid	Stearic acid		Emulsifier for food and no bioactivity [19]
Bis(2-ethylhexyl) phthalate			Cardio toxic effects, hepatotoxic [26]
2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-he			Not found
9(11)-Dehydroergosterylbenzoate	Monocarboxylic acid		Anti-inflammatory and anti-allergy (PubChem CID 637039)
9(11)-Dehydroergosterolsylate			Not found
4,6-Decadienal,8-ethyl-10-[4-hydroxy-8-(2			Abortifacient, Allergenic [19]

IV. DISCUSSION

Mushrooms have made its way into many homes from time immemorial as food due to its nutritional value, taste and aroma and can easily take the place of meat in a

vegetarian dish. Some (both edible and non-edible) mushrooms have been exploited for use in traditional medicine. In recent time, the medicinal uses of mushrooms and other plants have been studied but their bioactive compounds have been under-reported. The study being

reported here shows the compounds present in the acetone extracts of *D. elegans* to include but not limited to saturated fatty acids, aromatic acids, volatile aromatic hydrocarbons, alkane, anhydride, monocarboxylic acid and so on.

The antimicrobial resistance problem around the world and emerging diseases have made it a point to source for new therapeutic alternatives and higher fungi have shown to possess diverse activities that can be exploited both in pharmaceutical and industries. Some of the therapeutic activity possessed by this macrofungi include antimicrobial (antibacterial and anti-fungal), anti-viral, anti-inflammatory, cancer chemotherapy, antioxidant, hypocholesterolemia, anti-androgenic [23,24,25]. Other use include emulsifier for food [19] industrial mold release agents, production of soap, cosmetics and perfumes [25, 24, 27].

The GC-MS study carried out on the acetone extract of *D. elegans* in this study identified twenty-eight phytochemicals. There compounds identified are Benzoic acid (0.40%), Nonanoic acid (0.14%), Oxetane, 2,2,4-trimethyl- (0.28%), n-Decanoic acid (0.09%), Phthalimide (0.44%) Dodecanoic acid (0.24%), E-2-Hexenyl benzoate (0.21%), 2,4-Difluorobenzene, 1-benzyloxy- (0.16%), Tetratetracontane (0.55%), Isopropylphosphonic acid, fluoroanhydride (0.28%), Benzene, (1-methylundecyl)- (0.21%), Tetradecanoic acid (0.76%), Cyclohexanepropanol, .alpha.,2,2,6-tetrame (0.56%), Pentadecanoic acid (0.71%), E-2-Hexenyl benzoate (0.32%), Pentadecanoic acid (0.97%), 1-Decanol, 2-hexyl- (0.46%), 9-Tetradecenal, (Z) (1.67), n-Hexadecanoic acid (23.59%), Phthalic acid, butyl undecyl ester (1.08%), Eicosanoic acid (0.79%), 9,12-Octadecadienoic acid (Z,Z)- (44.64%), Octadecanoic acid (6.98%), Bis(2-ethylhexyl) phthalate (2.64%), 2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol (1.95%), 9(11)-Dehydroergosteryl benzoate (8.37%), 9(11)-Dehydroergosteroltosylate (1.28%), 4,6-Decadienal, 8-ethyl-10-[4-hydroxy-8-(2-hydroxypropyl)-3,9 (0.22%).

The acetone extract of *D. elegans* shows that it is rich in saturated fatty acids, Aromatic acid, Volatile organic compound, Alkane, Anhydride, Volatile aromatic hydrocarbon and Monocarboxylic acid, all of which possess both pharmaceutic and therapeutic activities as well as great and varied use in textile, paint, food and cosmetic industries.

Saturated fatty acids are either short-chain containing 4–12 carbon chains, mid-chain containing 13–16 carbon long chains and long-chain fatty acids of 17–26 carbon chains. Saturated fatty acids are important to nutrition because of

their ability to elevate blood lipid levels in humans. Next to trans fatty acids that are produced as a result of hydrogenation of polyunsaturated fatty acids, saturated fatty acids are the fatty acids with the greatest blood lipid elevating effect in humans. The omega-6 and omega-3 are essential fatty acids which are not produced by the body. They help in maintaining the cell membrane and control nutrient use along with metabolism. If we consume a meal with unsaturated fat, the glucose and other nutrients will directly rush into the bloodstream without being absorbed. Whereas if there is an intake of saturated fat, digestion will slow down and body will get more time to absorb the energy and nutrients from the meal.

Alkanes are important raw materials of the chemical industry and the principal constituent of gasoline and lubricating oils. It as well has an important role in human metabolism (PubChem CID 23494). Many aromatic compounds are used as solvents to remove or thin out oil- or grease-based compounds. Toluene, for example, is an ingredient in paint thinners. Benzene is a gasoline additive that reduces knocking in engines. Benzene and toluene are widely used to make other chemicals including dyes and plastic products.

Carboxylic acid on the other hand finds its use in manufacturing of soaps. Soaps are generally sodium or potassium salts of higher fatty acids such as stearic acid. Food industry uses many organic acids for the production of soft drinks, food products etc. For example, acetic acid is used in making vinegar. Sodium salts of organic acids find application in preservatives. The benefit of pharmaceutical industry from carboxylic acids and its derivatives includes its use in many drugs such as aspirin, phenacetin etc. It is a solubilizer acting in modulating solubility, lipophilicity, and cell permeation (e.g., antibiotic or antihistaminic drugs). It also serves as Prodrug or bio-precursor acting as compounds not biologically active but converted into active ones in specific conditions such as drugs from antihypertensive, antithrombotic, or antiviral. Carboxylic acid-containing drugs play a major role in the medical treatment of pain and diseases [28].

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